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Review

Macrolides beyond the conventional antimicrobials: a class of potent immunomodulators

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Abstract

The historical change in the natural course of diffuse panbronchiolitis (DPB), a fatal disorder of the airways, following the introduction of erythromycin in its treatment has focused attention of researchers on the anti-inflammatory properties of macrolides. Chronic inflammation of the airways accompanied by infiltration by neutrophils and overproduction of mucus and pro-inflammatory cytokines is observed in bronchial asthma, cystic fibrosis (CF), DPB, chronic obstructive pulmonary disease (COPD) and bronchiectasis. The airways of these patients are often colonised by mucoid *Pseudomonas aeruginosa* attached to epithelium by a biofilm. Bacteria intercommunicate for biofilm formation by a system of lactones known as quorum sensing. Macrolides inhibit mobility and quorum sensing of *P. aeruginosa*; they also decrease production of mucus by epithelial cells and biosynthesis of pro-inflammatory cytokines from monocytes and epithelial cells by inhibiting nuclear factor-κB. Large, randomised clinical trials for the management of these disorders with macrolides are not available, with the sole exception of four trials denoting benefit following long-term administration of azithromycin in patients with CF. That benefit is consistent with an increase in forced expiratory volume in 1 s (FEV_1) and a decrease in the rate of bacterial exacerbations. Studies with small numbers of patients with COPD revealed attenuation of the inflammatory reaction by macrolides. Experimental studies of Gram-negative sepsis have shown considerable attenuation of the systemic inflammatory response following intravenous administration of clarithromycin. Results of the effects of clarithromycin in patients with ventilator-associated pneumonia and sepsis in a large, randomised study of 200 patients are awaited.

Keywords: Macrolides; Inflammation; Cystic fibrosis; Bronchial asthma; Diffuse panbronchiolitis; Sepsis

1. Introduction

Macrolides are an old class of antimicrobials with an antimicrobial spectrum against mainly Gram-positive cocci and atypical pathogens. However, there is an accumulating body of evidence over the last few years that part of the activity of macrolides is not mediated through their traditional antimicrobial effect. This belief was created by the use of macrolides for the management of diffuse panbronchiolitis (DPB). DPB is a chronic devastating disorder solely presenting in Eastern Asian populations. The disorder is characterised by chronic inflammatory infiltration of the bronchi accompanied by chronic respiratory failure and cor pulmonale. The 5-year survival rate was <50% until 1979 when erythromycin was introduced as the main treatment, leading to considerable prolongation of survival and subsequent reduction of the death rate [1]. These patients have airway colonisation by *Pseudomonas aeruginosa*, which often leads to repeated cycles of exacerbation and remission. *Pseudomonas aeruginosa* does not belong to the antimicrobial spectrum of macrolides, raising numerous questions about the mechanism of action in DPB.

The anti-inflammatory action of macrolides is the most probable explanation for this phenomenon [2]. This belief is further reinforced by clinical data showing that the outcome of pneumococcal infections is favourable when macrolides are included in the treatment regimen. More precisely, retrospective analysis of cases of bacteraemia caused by *Streptococcus pneumoniae* revealed that addition of a macrolide to a β-lactam decreased the relative risk of death by 2.5-fold [3]. In the same context, a retrospective analysis of a Spanish series of patients with pneumococcal pneumonia showed lower mortality among those treated with a combination of a β-lactam and a macrolide compares with β-lactam monotherapy [4]. These observations have

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focused the attention of researchers to probable interactions of macrolides with the host immune system.

Despite increased knowledge about the non-antimicrobial activity of macrolides, clinical data are either missing or are restricted to studies with a limited number of patients. The present review discusses the significance of the non-antimicrobial activity of macrolides for the management of human disease. It is divided into three parts: in the first part, the effect of macrolides on cells of the immune system is analysed; in the next two parts, clinical data regarding the immunomodulatory role of macrolides for human disease are presented, with special emphasis on diseases of the lower respiratory tract.

2. What is the anti-inflammatory mode of action of macrolides?

Although DPB is an entity uncommon in Western societies, it has been used as a prototype of chronic inflammation of the airways, with particular attention on the role of colonisation by *P. aeruginosa* as a factor eliciting exacerbations. As a consequence, it is hypothesised that a favourable outcome following macrolide treatment is probably connected to an effect on the host–bacteria interaction.

2.1. Effect of macrolides on P. aeruginosa

Pseudomonas aeruginosa is a species with natural resistance to many antimicrobials, including macrolides. The bacterium exists in two forms, a planktonic form that is motile with a single polar flagellum and a sessile form through which bacteria attach to abiotic surfaces or organic substances leading to biofilm formation. It is conceived that these biofilms protect bacteria from antibiotics, complement and opsonising antibodies [5,6]. *Pseudomonas aeruginosa* is also equipped with type IV pili, which confer twitching motility once bound to smooth surfaces and to disaccharides of the bronchi. These pili allow single bacterial cells to attach to each other and contribute to the generation of biofilms. The minimum inhibitory concentration (MIC) of macrolides for *P. aeruginosa* is very high, usually exceeding 500 μg/mL. Clarithromycin at very low concentrations equal to 0.03× MIC is not able to reduce the production of type IV pili by the bacteria. However, using electronic microscopy it was evident that clarithromycin inhibits the assembly of pili, thus restricting twitching motility and the subsequent formation of biofilm [7]. Fluorescent confocal microscopy showed that azithromycin at concentrations of 2 μg/mL retarded the formation of biofilm by *P. aeruginosa* PA01 [8].

Biofilm formation and virulence factors in *P. aeruginosa* are controlled by a system of bacterial intercommunication, known as quorum sensing (QS). This system comprises genes encoding transcriptional activators, namely *lasR* and *rhlR*, and genes encoding autoinducer synthases, namely *lasI* and *rhlI*. The latter are required for the biosynthesis of the autoinducer molecules 3-oxo-C_{12}-homoserine lactone (HSL) and C_4-HSL [9]. Recently, QS was shown to involve many more genes involved in the regulation of virulence factors in *P. aeruginosa*. Macrolides inhibit the transcription of several of these genes [10,11]. More precisely, azithromycin at concentrations of 2 μg/mL reduces the transcription of *lasI* by 80% and *rhlI* by 50%, leading to a subsequent reduction in the production of 3-oxo-C_{12}-HSL and of C_4-HSL by 94% and 72%, respectively [12]. The effect of azithromycin on *P. aeruginosa* is accompanied by reduced twitching motility and by an increased susceptibility of the bacteria to H_2O_2, i.e. to phagocytosis [11]. Biofilm formation mimicking chronic infection has been reproduced in a murine model after intubation with a plastic tube pre-coated by the bacteria. Oral treatment with a combination of clarithromycin and levofloxacin or with high doses of clarithromycin reaching 100 mg/kg significantly reduced the number of bacteria in lungs [13,14].

2.2. Effect of macrolides on the host (Fig. 1)

2.2.1. Effect on bronchial cells

Knowledge acquired by the efficacy of long-term treatment with erythromycin in patients with DPB created the question of whether macrolides may directly interfere with the effect of *P. aeruginosa* on epithelial cells of the airways. Flagellin is an outer membrane protein and a potent inducer of pro-inflammatory phenomena comprising the biosynthesis of interleukin (IL)-8 by normal human bronchial epithelial (NHBE) cells [15]. Part of its activity is thought to be mediated through the phosphorylation of extracellular signal-regulated kinase (ERK). Addition of 10 μg/mL clarithromycin affects the in vitro production of IL-8 by NHBE cells by a mode characterised by two distinct phases; in the early phase of addition of clarithromycin production of IL-8 is decreased, but with time it is increased to 154% of the baseline. This is accompanied by changes in the degree of phosphorylation of ERK [16,17]. The authors proposed that the rebound in the production of IL-8 with the progression of time might be connected to crosstalk of intracellular pathways or to negative feedback. By an ERK-mediated process, clarithromycin suppresses growth of NHBE cells and delays their transition from phase G_1 to phase S of the cell cycle [18].

Further in vitro studies using NHBE cells revealed that azithromycin increases transepithelial electrical resistance. The working hypothesis was that tight junctions between epithelia of the airways fail in DPB and cystic fibrosis (CF), with considerable consequences on the electrolyte content of the airway surface liquid. The favourable action of azithromycin was mainly shown with concentrations of 40 μg/mL. Its mode of action involved the transition of claudin-1 and -4 and occludin to an intracellular location, thus better regulating tight junctions between epithelia. The effect of azithromycin was fully reversible upon removal from cell culture [19].

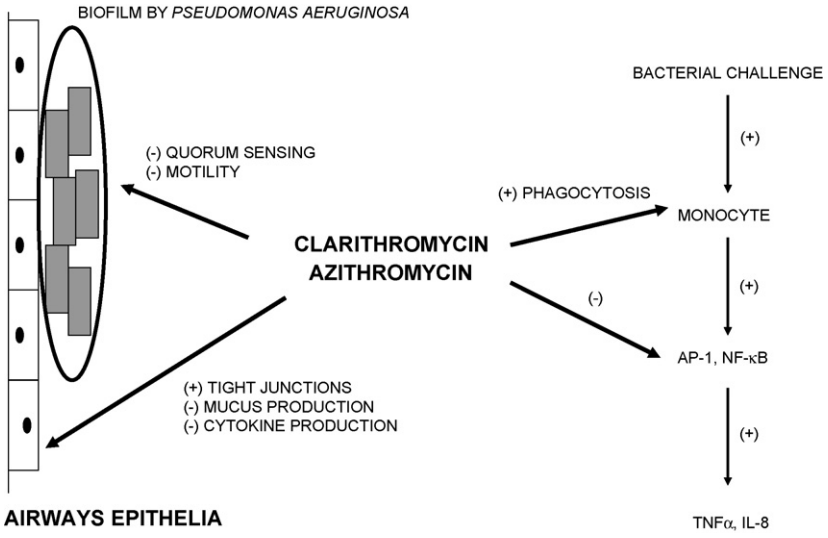

Fig. 1. Schematic representation of the anti-inflammatory mode of action of macrolides. –, inhibition; +, stimulation; AP-1, activator protein-1; NF-κB, nuclear factor-κB; TNFα, tumour necrosis factor-alpha; IL-8, interleukin-8.

2.2.2. Effect on the immune system

Implications for a role of macrolides in the function of either the innate or the adaptive immune system derive from clinical studies of patients with community-acquired pneumonia. Comparison between 23 patients orally treated with clarithromycin 500 mg twice a day (bid) for 7 days and 23 patients orally treated with amoxicillin 1 g three times a day for 7 days was accompanied by considerable changes over follow-up compared with baseline of the serum cytokine levels in the clarithromycin treatment arm but not in the amoxicillin-treated population. These changes comprised decrease of the pro-inflammatory cytokine IL-6, increase of the anti-inflammatory IL-10 and increase of interferon-gamma (IFNγ) [20].

These changes are probably related to an effect of macrolides on blood monocytes [21]. More precisely, clarithromycin inhibits the in vitro production of IL-6, IL-8 and macrophage inflammatory protein (MIF)-2 by human monocytes, the human monocytic cell line THP-1 and the murine RAW264.7 macrophage cell line following stimulation either by lipopolysaccharide (LPS) or by a lysate of *Escherichia coli* or *P. aeruginosa* [22,23]. Its action is mainly shown at concentrations close to 10 μg/mL and is related to inhibition of the transcription regulators nuclear factor (NF)-κB and activator protein (AP)-1 [22]. At similar concentrations, clarithromycin suppresses the release of superoxide from mast cells [24].

The effect of macrolides on the cells of the innate immune system is not only limited to modulation of the production of pro- and anti-inflammatory mediators but is also expanded to the effector mechanism of phagocytosis. More precisely, 14- and 15-member macrolides such as clarithromycin and azithromycin increase the in vitro rate of phagocytosis of sequestered apoptotic neutrophils and apoptotic bronchial epithelial cells by alveolar macrophages [25,26]. This effect is shown with alveolar macrophages isolated both from healthy volunteers and from patients with chronic obstructive pulmonary disease (COPD). However, induction of phagocytosis was less effective in the latter population. The action of macrolides is probably mediated, at least in part, through the phosphatidylserine (PS) receptor because it is inhibited upon addition of liposomes that bind the PS receptor [26].

The above in vitro data clearly signify the existence of a direct effect of macrolides on the function of cells participating in the innate host defence. Recent evidence suggests a similar effect on cells of the adaptive immune system. This involves both the process of antigen presentation and T-cell regulation. Antigen presentation in the host is mediated through dendritic cells (DCs), which are widely distributed in all tissues except the brain. Expression of the CD80 surface marker on DCs isolated from the bone marrow of BALB/c mice was primed by azithromycin or clarithromycin but not by the 16-member macrolide midecamycin. When DCs were co-cultured with T-lymphocytes of the same mice and stimulated by LPS, it was found that clarithromycin suppressed the in vitro production of IL-6 and that azithromycin induced the biosynthesis of IL-10 [27].

T-helper function is clearly distinguished into Th1 and Th2 responses. The former is characterised by secretion of IFNγ and favours a cell-mediated immune response, whilst the latter is characterised by secretion of IL-4 and favours humoral immunity. At concentrations similar to those affecting the function of monocytes, clarithromycin inhibits intracellular production of IL-4 by T-helper lymphocytes leading to an increase of the Th1/Th2 ratio [28].

3. Role of macrolides for the management of chronic inflammatory disorders of the lower respiratory tract

The clinical significance of the above-mentioned in vitro anti-inflammatory effects of macrolides is mainly dependent

on their pharmacokinetics with special emphasis on their penetration to epithelial lining fluid (ELF) and alveolar macrophages. This is the salient factor determining the probability of macrolides behaving as immunomodulators for chronic disorders of the lower respiratory tract. Concentrations of clarithromycin in ELF following a single oral dose of 500 mg range between 15 μg/mL and 72 μg/mL; intracellular concentrations in alveolar macrophages are almost 100-fold higher than in serum [29,30]. Similar intracellular levels of azithromycin are reported after single oral doses, whereas those in ELF are <1 μg/mL [31,32].

These data render favourable the administration of macrolides for chronic disorders of the airways, particularly patients with asthma, CF, DPB and COPD.

3.1. Macrolides in bronchial asthma

Asthma is a chronic inflammatory disorder of the airways characterised by bronchial hyperresponsiveness and paroxysmal attacks of wheezing. Therapy with macrolides is considered beneficial for patients owing both to their anti-inflammatory and antibacterial properties. Macrolides decrease infiltration of the airways by eosinophils and the production of eotaxin and RANTES (regulated upon activation, normal T-cell expressed and secreted) by these cells [33]. However, it is reported that many asthmatic patients bear chronic infection by *Chlamydia pneumonia* and *Mycoplasma pneumoniae* against which macrolides are active [34].

Several double-blind, placebo-controlled clinical studies have been performed in a limited number of patients to evaluate the effect of various macrolides in patients with asthma. In three patients, clarithromycin 500 mg bid for 12 months decreased requirement for prednisone in prednisone-dependent asthma [35]. In a double-blind, placebo-controlled study of 21 patients with corticosteroid-dependent asthma, it was later found that clarithromycin 500 mg bid for 6 weeks increased the forced expiratory volume in 1 s (FEV_1) and decreased nocturnal dyspnoea [36]. A similar study with 52 patients disclosed benefit of that dose regimen of clarithromycin only for those who were polymerase chain reaction-positive for *Chlamydia* or *Mycoplasma* [37]. In a recent trial, 75 patients were randomly allocated to receive either placebo or clarithromycin 250 mg bid for 8 weeks or clarithromycin 500 mg bid for 8 weeks. Evaluation was based on the provocative dose of methacholine causing a 20% fall (PD_{20}) in FEV_1 at baseline and 24 h after completion of therapy with the study drug. PD_{20} was significantly increased in both clarithromycin-treated groups of patients [38]. Finally, 16 children with asthma were randomly assigned to either placebo or 10 mg/kg oral azithromycin for 3 days per week for a total of 8 weeks. At the end of treatment with azithromycin, FEV_1 after hypertonic saline inhalation decreased considerably, as did neutrophils in sputum. Similar changes were not found in placebo-treated children [39].

The largest randomised trial comprised 278 patients with acute exacerbation of asthma assigned to oral telithromycin 800 mg once a day (qd) or placebo for 10 days. Administration of telithromycin was accompanied by earlier resolution of symptoms and significant increase of FEV_1 from baseline. The effect on FEV_1 at the end of treatment was lost after 1 month. Surprisingly, benefit was mainly shown for patients with positive serology for an acute infection by either *M. pneumoniae* or *C. pneumoniae*, pointing towards a direct antibacterial effect as the proposed mode of action of telithromycin [40].

3.2. Macrolides in CF

The efficacy of erythromycin in DPB also turned the interest of researchers to the efficacy of macrolides for the treatment of patients with CF. CF is a genetically inherited disorder due to mutation of the chloride channel protein, also called cystic fibrosis transmembrane regulator (CFTR). These mutations change the composition of electrolytes in the extracellular fluid surrounding the epithelia of the airways thus making favourable chronic colonisation by mucoid *P. aeruginosa* and recurrent bacterial exacerbations. Several non-randomised trials have shown a benefit from long-term azithromycin intake as an increase of FEV_1 and the improvement of respiratory function of patients [41,42]. That created the need for randomised, placebo-controlled trials. Since 2002, results of four trials comparing placebo with azithromycin have been published and their results are summarised in Table 1. Azithromycin was administered for a period of 3–12 months. All trials disclosed a considerable effect of azithromycin in the improvement of lung function as assessed by increase of FEV_1 from baseline. Furthermore, treatment with azithromycin was related to a reduced risk of bacterial exacerbations and a reduced need for antimicrobial use [43–46].

The underlying mechanism of action of azithromycin in these patients is a matter of debate. Proposed hypotheses are based on the reported in vitro evidence for the anti-inflammatory mode of action of macrolides, which may involve any of the following sites: *P. aeruginosa*; airway epithelial cells; and modulation of cytokine response (Fig. 1). The results of various studies on the mechanism of action of azithromycin are characterised by considerable discrepancies.

In vitro studies with 13 isolates of *P. aeruginosa* from patients with CF showed that azithromycin at sub-MIC concentrations reduced bacterial adherence to bronchial mucin. However, the effect of azithromycin varied from one isolate to another [47]. This in vitro observation was verified after analysis of *P. aeruginosa* isolates collected from the sputa of patients enrolled in one of the randomised clinical trials of the efficacy of azithromycin in CF [46]. The increase of FEV_1 found in the azithromycin treatment arm was connected to decreased production of phospholipase C by the isolates [48].

However, the most recent of the four randomised trials disclosed the best clinical benefit of azithromycin among

Table 1
Summary of four randomised clinical trials of the effect of azithromycin (AZM) in patients with cystic fibrosis

Reference	Dose regimen	No. of patients analysed	Effect on lung function	Secondary outcomes	Adverse events
Wolter et al. [43]	250 mg qd × 3 months	Placebo = 30; AZM = 29	Increase of FEV_1	↓ days of i.v. treatment for bacterial exacerbations; ↓ hospital days; improvement of dyspnoea	Urticaria 1 patient; rash 1 patient; neutropenia 1 patient
Equi et al. [44]	250 mg or 500 mg qd × 6 months	Crossover after 1 month washout	Increase of FEV_1	No benefit in quality of life	↑ transaminases 1 patient
Saiman et al. [45]	250 mg or 500 mg 3 times/week × 168 days	Placebo = 97; AZM = 87	Increase of FEV_1	↓ risk for bacterial exacerbations; gain of body weight; improvement in physical condition	Nausea (17%); diarrhoea (15%); wheezing (13%)[a]
Clement et al. [46]	250 mg or 500 mg 3 times/week × 12 months	Placebo = 37; AZM = 35	Increase of FEV_1	↓ risk for bacterial exacerbations	No differences to placebo

qd, once daily; FEV_1, forced expiratory volume in 1 s; i.v., intravenous; ↓, decrease; ↑, increase.
[a] Percentages in parenthesis refer to increase over placebo.

patients who were not colonised by *P. aeruginosa* [46]. This result is against the hypothesis that favourable outcome by azithromycin is attributed to an effect on the biology of *P. aeruginosa*. In accordance with that observation are the results of an open-label trial with nine CF patients administered 500 mg azithromycin daily for 2 weeks. At the end of treatment, no effect was observed on bacterial adherence to epithelial cells [49]. In the latter group of patients, nasal epithelial cells were collected after brushing at baseline and at the end of treatment to estimate levels of mRNA of the gene encoding for CFTR as well as potential difference, which is an indirect index of ion transport. Azithromycin had no effect on either of these two parameters.

In a recent randomised clinical trial, 18 patients with CF were randomly allocated to receive either placebo or clarithromycin 500 mg daily for 5 months. Crossover was done after a washout period of 1 month. The endpoint of the study was to evaluate the mechanism of action of clarithromycin in CF after estimation of potential difference in nasal epithelial cells collected after brushing. Clarithromycin was of no proven benefit [50].

The above findings point towards a modulating effect of macrolides on the production of cytokines by epithelial cells of the airways or by cells of the immune system as the most probable underlying mechanism of action. In a recent open-label trial, 250 mg clarithromycin every other day was administered in 27 patients with CF. Concentrations of tumour necrosis factor-alpha (TNFα), IL-4 and IL-8 in sputum were decreased and concentrations of IFNγ were increased compared with baseline at 3, 6 and 12 months follow-up. Administration of clarithromycin was also accompanied by an increase of the Th1 to Th2 response [51]. In support of this clinical observation, addition of azithromycin to cultures of epithelial cells bearing the CF phenotype decreased mRNA of TNFα and IL-8, but not of IL-6. Its effect was connected to inhibition of AP-1 and NF-κB [52,53].

3.3. Macrolides in DPB

DPB is a chronic inflammatory disorder with a mortality rate >50% after 10 years of follow-up. Airways are chronically infected by mucoid *P. aeruginosa* leading to a life with continuous bacterial exacerbations and remissions, with respiratory failure and cor pulmonale as a final outcome [54]. A retrospective analysis of 500 cases showed that the survival rate was increased to almost 90% after 1984 when erythromycin was introduced as empirical therapy. Later, erythromycin was replaced by clarithromycin. At a daily dose of 100 mg for 8 weeks, clarithromycin decreased overproduction of sputum by patients [55]. In a recent open-label, one-arm study by Kadota et al. [56], 200 mg clarithromycin was administered orally each day for 4 years. Patients were followed-up by spirometry and estimation of blood gases. Compared with their baseline values, significant increases of FEV_1 and vital capacity were found in all patients accompanied by similar changes in partial pressure of oxygen (pO_2). The effect was found as early as 3 months after the start of therapy and lasted for the entire 4-year study period.

The mechanism of action of clarithromycin in DPB is based either on a direct effect on the function of the airway epithelia or on *P. aeruginosa*. Epithelia of patients with DPB overproduce mucin, where the protein muc5ac constitutes its basic core. In an animal model of DPB, production of muc5ac was reduced following oral treatment with clarithromycin [57].

3.4. Macrolides in bronchiolitis obliterans

Bronchiolitis obliterans is the leading cause of death after lung transplantation. Azithromycin was administered orally at 250 mg three times a week for 3 months along with conventional immunosuppressive treatment in 14 patients who underwent lung transplantation. A considerable increase in FEV_1 was found in six patients; neutrophils and mRNA of

IL-8 were decreased in fluid collected after bronchoalveolar lavage (BAL) from all patients [58].

Clarithromycin was administered in a case series of six patients presenting with cryptogenic pneumonia; three had a history of malignancy. The dose regimen varied between 250 mg and 500 mg bid for 10 days to 6 weeks. An excellent response of symptoms was denoted in five patients along with signs of radiographic resolution [59].

3.5. Macrolides in COPD and bronchiectasis

COPD is characterised by progressive development of airflow limitation by the airways, which is not fully reversible [60]. This is accompanied by infiltration of the airways by neutrophils and mucus hypersecretion. Experimental studies have shown that oral administration of macrolides reduces accumulation of neutrophils and subsequent production of mucin. Administration of clarithromycin in a rat model after allergic sensitisation of the nasal epithelium accompanied by challenging with LPS revealed a considerable inhibition of hypertrophic and metaplastic changes of goblet cells in clarithromycin-treated rats compared with controls [61].

In a recent double-blind study, 27 patients with COPD were randomised in a 2:1 ratio either to azithromycin 500 mg qd for 3 days or to placebo. Administration of azithromycin was accompanied by considerable changes in serum cytokines and indexes of degranulation as well as the oxidative burst of neutrophils that lasted up to 15 days after the end of treatment. These changes involved decreases of serum IL-8 and lactoferrin and of β_2-microglobulin of neutrophils [62]. In the same context, a prospective double-blind study enrolled 30 patients with COPD; 15 were placebo-treated and 15 were treated orally with clarithromycin 500 mg bid for 15 days. Concentrations of TNFα and IL-8 in sputum were significantly decreased post treatment in the clarithromycin arm; no changes in the pulmonary function tests were found in the latter arm of treatment [63].

The influence of oral treatment with clarithromycin was also tested in a randomised trial of 34 children with bronchiectasis. Children with CF and primary immunodeficiencies were excluded from this study. Clarithromycin was administered in 17 of them at a dose of 15 mg/kg for 3 months. The main outcomes of this study were a significant reduction of the neutrophil count and of IL-8 in BAL at the third month demonstrated only among patients treated with clarithromycin but not among those treated with placebo [64]. The volume of sputum was also decreased but that change was too close to statistical significance ($P = 0.06$). Finally, clarithromycin had no effect on FEV_1.

4. Do macrolides have any role for the management of acute inflammatory conditions?

The data already analysed implicate a direct anti-inflammatory effect of macrolides for chronic inflammatory disorders of the airways. According to these data, macrolides should be administered as long-term therapy. The question arising is whether macrolides could be administered for the management of acute inflammatory disorders. The prototype of these conditions is systemic inflammatory response syndrome in the setting of an acute infection, i.e. sepsis. Available data for a possible role of macrolides in sepsis are limited to experimental models.

Most of these experimental data are based on an experimental model of acute pyelonephritis in rabbits described by our group with intravenous (i.v.) clarithromycin as an immunomodulatory therapy. The aim of these studies was to achieve with i.v. administration serum concentrations of clarithromycin close to 10 μg/mL, which has been reported as the most potent in inhibiting NF-κB in vitro [22]. Clarithromycin was administered in rabbits with acute pyelonephritis caused by multidrug-resistant *P. aeruginosa* [65,66], susceptible *E. coli* [67,68] and pandrug-resistant *Klebsiella pneumoniae* [69]. Therapy of the animals was started either in parallel with bacterial challenge or upon presentation of signs of sepsis. Clarithromycin was given alone or in combination with amikacin. Documented signs of sepsis were pulmonary oedema observed 6 h after challenge by *E. coli*, and hypothermia, tachycardia and leukocytosis after challenge by *P. aeruginosa*. In all cases, co-administration of clarithromycin and amikacin prolonged survival; in studies of sepsis by *E. coli* favourable outcome was also shown with clarithromycin alone.

Modulation of the systemic inflammatory response by clarithromycin has been proposed as the most probable explanation for the observed clinical benefit. In vitro, the administered agents did not have any effect on the test pathogens. Replication of the test isolate remained unchanged in the site of infection in all studies. All tested animals (controls and treated) had the same degree of bacteraemia and endotoxaemia, excluding any probability for a direct antibacterial effect as a mode of action. Serum TNFα and oxidant status were significantly decreased among clarithromycin-treated animals. Blood monocytes were implicated as the most probable site of the immunomodulatory action of the drug. Following treatment with clarithromycin, monocytes presented lower ex vivo secretion of TNFα and decreased intracellular activity of caspase-3, signifying that early apoptosis of blood monocytes was connected with a favourable outcome [70]. Inflammatory infiltration of the liver, lung and spleen was decreased in clarithromycin-treated rabbits compared with controls as assessed both by the density of neutrophils and lymphocytes and maintenance of the architecture of organs. Bacterial load did not differ between clarithromycin-treated animals and controls [66,69]. In a recent study of lethal shock induced in mice following intraperitoneal challenge with LPS, oral pre-treatment with azithromycin decreased lethality without altering the degree of tissue infiltration by neutrophils [71].

Regarding the favourable immunomodulatory effect of clarithromycin as described from the experimental studies

on Gram-negative bacteria, it should be underscored that this was observed even when therapy was started late in the course of sepsis. On that basis, a double-blind, placebo-controlled, multicentre study was performed to evaluate the safety and efficacy of intravenously administered clarithromycin in 200 patients with septic syndrome due to ventilator-associated pneumonia. The drug was administered at a dose of 1 g qd by 1-h infusion for 3 consecutive days. The study is registered (http://www.clinicaltrials.gov; NCT00297674) and recruitment has been ended. Results have not yet been published.

Although results of this trial are expected to demonstrate clearly the existence of benefit, if any, of clarithromycin in acute inflammatory conditions, implications for the clinical effect of macrolides is derived indirectly. More precisely, it has been described from retrospective analysis of a cohort of patients with pneumococcal bacteraemia that addition of a macrolide to a β-lactam significantly decreased the risk of death [3]. Similar results are found from a recent trial in 30 infants with acute bronchiolitis by respiratory syncytial virus. Patients were randomised to receive either placebo or 15 mg/kg clarithromycin daily for 3 weeks. Nine infants were excluded from final analysis because they needed treatment with steroids. Statistical analysis included 9 placebo-treated and 12 clarithromycin-treated infants. Administration of clarithromycin significantly reduced length of stay, duration of supplemental oxygen requirement and i.v. fluids and duration of β_2-agonist therapy compared with the placebo group. This was accompanied by decreased serum levels of IL-4, IL-8 and eotaxin [72].

5. Conclusions

The above review of all recent data in the literature clearly describes macrolides as agents that, far beyond their traditional antimicrobial properties, possess a considerable anti-inflammatory effect (Fig. 1). Their exact role in everyday clinical practice for the therapy of chronic respiratory inflammatory conditions depends on results of large randomised clinical trials. With the sole exception of four trials disclosing considerable benefit from the long-term administration of azithromycin in patients with CF (Table 1), these trials are missing for bronchial asthma, COPD and bronchiectasis. Another dilemma arising from the long-term administration of macrolides is the risk for acquisition of resistance by the normal flora. A recent study enrolled a total of 224 healthy volunteers; 74 were randomised to receive 500 mg clarithromycin bid for 7 days, 74 azithromycin 500 mg qd for 3 days and 76 volunteers were equally distributed to receive placebo matching to clarithromycin and azithromycin. Samples were taken from the oropharynx to evaluate the advent of resistance in normal streptococcal flora. Macrolide-treated subjects acquired resistant flora at far higher percentages than placebo-treated volunteers. These were statistically higher among azithromycin-treated than clarithromycin-treated volunteers, being 51.8% vs. 38.3% ($P = 0.0010$) at Day 14 from baseline, 50.1% vs. 32.7% ($P < 0.0001$) at Day 28 from baseline and 35.5% vs. 25.7% ($P = 0.0200$) at Day 42 from baseline [73]. These results raise fear about the high probability of acquisition of macrolide resistance from long-term administration and verify prospective data associating administration of azithromycin in patients with CF with the isolation of erythromycin-resistant *Staphylococcus aureus* and *Haemophilus* spp. from their sputum [74,75]. Despite scepticism about the risk of resistance development, anti-inflammatory properties of macrolides add new perspectives regarding our armamentarium of agents capable of modulating the reaction of the host.

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